

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

KLIPPEL *et al.*

Application No.: Unassigned

Filed: Herewith

For: CONSTITUTIVELY ACTIVE
PHOSPHATIDYLINOSITOL 3-
KINASE AND USES THEREOF
(As Amended)

Examiner: Unassigned

Art Unit: Unassigned

PRELIMINARY AMENDMENT

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced application, please enter the following amendments and remarks.

IN THE SPECIFICATION

On page 1 at line 6 please add the following:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of, and claims the benefit of priority from, co-pending U.S. Patent Application Serial No. 09/265,772, filed March 10, 1999, which is a continuation of U.S. Application No. 08/390,874 filed February 17, 1995, now U.S. Patent No. 6,043,062, the full disclosures of which are incorporated herein by reference in their entirety.--

IN THE CLAIMS

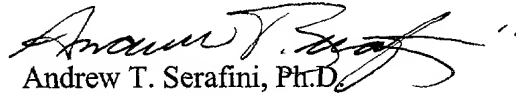
Please cancel claims 8-16 without prejudice. For ease of reference, claims pending with entry of this amendment are shown in the attached Appendix.

REMARKS

With entry of this Preliminary Amendment, claims 25-38 are pending in the application. Also, the specification has been amended to insert a reference to the priority applications. No new matter has been introduced.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned attorney at 650-326-2400 x 5228.

Respectfully submitted,


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Attachment: Appendix: Pending claims with entry of this amendment

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Appendix: Claims pending with entry of this amendment

25. A method of identifying a cellular target protein substrate of a phosphatidylinositol 3-kinase, said method comprising the steps of:

- (a) providing a phosphatidylinositol 3-kinase polypeptide of claim 1;
- (b) providing a test cell lysate thereof;
- (c) providing a negative control cell lysate not contacted with said phosphatidylinositol 3-kinase polypeptide;
- (d) contacting said phosphatidylinositol 3-kinase polypeptide with said test cell lysate in the presence of labeled ATP under conditions which allow said phosphatidylinositol 3-kinase to phosphorylate any cellular phosphorylated target protein; and
- (e) comparing said test cell lysate with said negative control lysate to detect said phosphorylated target protein in said test cell lysate and thereby identifying the cellular target protein substrate.

26. The method of claim 25, wherein the ATP is [$\gamma^{32}\text{P}$]ATP.

27. The method of claim 25, wherein both cell lysates are from stimulated cells.

28. The method of claim 25, wherein said phosphorylated target protein is detected by:

performing SDS-PAGE on said cell lysates after step (d) to separate proteins contained therein, on a gel matrix; and

preparing an autoradiograph of said gel to detect radiolabeled bands present in the test cell lysates, but absent from the negative control lysate, as indicative of the presence of said phosphorylated target protein in the test cell lysate.

29. A method of identifying an associating protein of an active phosphatidylinositol 3-kinase, comprising:

expressing a constitutively active phosphatidylinositol 3-kinase polypeptide of claim 1 or 7 in a mammalian cell under conditions which favor expression of the polypeptide;

biosynthetically labeling proteins of said active kinase expressing cell to produce labeled proteins;

obtaining a lysate from said cell;

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immunoprecipitating said expressed phosphatidylinositol 3-kinase polypeptide from said cell lysate to produce an immunocomplex;

detecting a labeled protein that co-immunoprecipitates with said phosphatidylinositol 3-kinase polypeptide, within said immunocomplex, wherein said co-immunoprecipitating protein is considered an associating protein.

30. The method of claim 29, wherein said co-immunoprecipitating protein is detected by solubilizing said immunocomplex to release labeled proteins contained therein; separating said released proteins by SDS-PAGE; and performing phosphatidylinositol 3-kinase polypeptide, said labeled protein being an associating protein.

31. A method of identifying an associating protein of an active phosphatidylinositol 3-kinase, comprising;

exposing a phage or bacterial peptide library to a constitutively active phosphatidylinositol 3-kinase polypeptide of claim 1 or 7 to allow binding of a peptide from said library to said constitutively active phosphatidylinositol e-kinase polypeptide to form a bound peptide; and

isolating said bound peptide, wherein said bound peptide is considered an associating protein.

32. The method of claim 31, wherein said bound peptide is isolated by affinity purification.

33. A method of screening for an inhibitor of phosphatidylinositol 3-kinase activity, said method comprising:

providing a constitutively active phosphatidylinositol 3-kinase polypeptide of claim 1 or 7;

exposing one or more test compounds to said phosphatidylinositol 3-kinase polypeptide in the presence of a phosphatidylinositol 3-kinase substrate and [³²P]ATP to allow phosphorylation of said substrate; and

assaying for the presence of phosphorylated substrate wherein the absence of phosphorylated substrate is indicative that the test compound is an inhibitor of phosphatidylinositol 3-kinase activity.

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34. The method of claim 33, wherein said substrate is phosphatidylinositol and said phosphorylated substrate is phosphatidylinositol 3'-P.

35. A method of treating a disease selected from the group consisting of proliferative, inflammatory, allergic and cardiovascular diseases, comprising administering to a patient, a therapeutic formulation comprising an inhibitor of phosphatidylinositol 3-kinase activity in an amount effective to block phosphatidylinositol 3-kinase activity in affected cells of said patient.

36. The method of claim 35, wherein the proliferative disease is cancer or psoriasis.

37. A method of promoting wound healing, comprising administering to a patient, a therapeutic formulation comprising a phosphatidylinositol 3-kinase polypeptide of claim 1.

38. A formulation for treating a proliferative disease, comprising a therapeutically effective amount of an inhibitor of phosphatidylinositol 3-kinase.

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